

Prevalence of *Helicobacter pullorum* among Patients with Gastrointestinal Disease and Clinically Healthy Persons

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Feces from 531 patients with gastroenteritis and from 100 clinically healthy individuals were tested for *Helicobacter pullorum* by use of PCR. Samples positive by PCR were qualified for isolation. *H. pullorum* DNA was demonstrated to be present in feces from 4.3% of patients with gastrointestinal disease but also in feces from 4.0% of clinically healthy persons. One strain was isolated from one patient with gastrointestinal disease.

Helicobacter pullorum is a gram-negative, slightly curved rod which is oxidase positive and negative for indoxyl acetate esterase, urease production, and hippurate hydrolysis. The organism is mostly catalase positive (16). *H. pullorum* requires a microaerobic environment supplemented with H₂ in order to grow (8, 16, 18, 20). Identification to the species level can be done on the basis of the phenotypic traits mentioned above, although the results of these tests are sometimes difficult to interpret. The correct identity of the species can be confirmed by means of PCR (18).

H. pullorum has, on several occasions, been detected in the ceca and on the carcasses of broiler chickens, in the intestines of laying hens, and in the feces of humans. DNA of this bacterial species has been demonstrated to be in the livers of laying hens and in the biliary trees of humans (5, 6, 8, 9, 10, 18, 20, 28). A number of research groups have associated this organism with vibronic hepatitis and enteritis in poultry and with gastroenteritis, diarrhea, and liver and gall bladder disease in human patients (8, 9, 18, 28). It has been suggested that *H. pullorum* also may play a role in Crohn's disease (1, 4).

The number of *H. pullorum* infections in people most probably has been and still is underestimated because of the phenotypic similarities between the genera *Helicobacter* and *Campylobacter* on the one hand and the specific isolation requirements of *H. pullorum* on the other hand (2, 10, 20, 28). Consequently, a significant number of patients with diarrhea may have been misdiagnosed in the past (2, 20, 28).

Despite the increasing number of reports stating that *H. pullorum* is a significant food-associated human pathogen, there is a total lack of information on the prevalence of this species in human beings.

The aim of the present study was to determine the prevalence of *H. pullorum* in human patients with gastrointestinal disease. For comparative purposes, clinically healthy persons were likewise included.

Five hundred thirty-one fecal samples were obtained from patients with gastrointestinal disease at the Department of Gastroenterology, University Hospital of Ghent, Ghent, Belgium, on an anonymous basis before being analyzed. One hundred fecal samples were collected on an anonymous basis from clinically healthy volunteers. All samples were stored at –20°C and –70°C for PCR and isolation, respectively, until further analysis.

DNA was extracted from feces (weighing approximately 200 mg) by use of a commercial stool kit (QIAamp DNA stool mini kit; QIAGEN, Venlo, The Netherlands). A PCR assay amplifying a 447-bp fragment of the 16S rRNA gene of *H. pullorum* was used (18). The PCR assay and gel electrophoresis of the PCR products were performed as previously described (3).

All samples positive by the PCR were qualified for isolation. The samples (200 mg) for isolation were put in a 1.5-ml tube with 400 µl of a mixture composed of 7.5 g glucose, 25 ml brain heart infusion (BHI) (Oxoid, Basingstoke, England), and 75 ml sterile inactivated horse serum and homogenized. The various samples were inoculated on BHI agar supplemented with 10% horse blood, amphotericin B (20 µg/ml) (Fungizone; Bristol-Myers Squibb, Epernon, France), and Vitox (Oxoid). The filter technique of Steele and McDermott (19) was used. Incubation was done in microaerobic conditions (5% H₂, 5% CO₂, 5% O₂, and 85% N₂) at 37°C for a minimum of 3 days. Very small, grayish white, hemolytic colonies were selected and purified on a BHI agar blood plate. The colonial morphology and phenotypic characteristics (gram negative, slightly curved rod, catalase and oxidase positive, and indoxyl acetate esterase negative) of the isolates were used for presumptive identification. Confirmation of the presumed identity was done on the basis of PCR and sequencing of the 16S rRNA gene as described below.

The PCR product of the retrieved *H. pullorum* isolate was purified and sequenced as described by Baele et al. (3). By use of BLAST software, sequences were compared to published *H. pullorum* 16S rRNA sequences obtained from GenBank (accession numbers AY631956, L36143, and L36144).

Twenty-three out of the 531 fecal samples (4.3%) from patients with gastrointestinal disease were found to be positive

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for *H. pullorum* in the PCR. In the group from clinically healthy individuals, 4 out of the 100 samples harbored *H. pullorum* DNA. *H. pullorum* was isolated from the feces of one patient with gastrointestinal disease. The sequence of the amplified 447-bp fragment of the *H. pullorum* 16S rRNA gene of this isolate revealed a similarity of 99% to those from GenBank.

The present study shows that fecal material from 4.3% of patients with gastrointestinal disease, but also that from 4.0% of clinically healthy persons, harbors *H. pullorum* DNA. The percentages in both groups may call into question the presumed association of *H. pullorum* with gastrointestinal and liver disease. One could indeed state that *H. pullorum* may reside in the normal gut floras of human beings. Its presence in fecal samples might also be passive, representing acquisition from contaminated food without replication of the organism in the intestinal tract. Hence, the possibility that the detection of *H. pullorum* in human feces might be an accidental finding cannot be excluded.

Several authors have, however, related *H. pullorum* to gastroenteritis resulting in diarrhea and liver and gall bladder disease in humans (5, 9, 20). Encountering *H. pullorum* DNA in feces from patients with gastrointestinal diseases as well as from clinically healthy individuals does not necessarily mean that this microorganism is not pathogenic. Indeed, predisposing factors which are hitherto unknown may cause some of the *H. pullorum* strains to make the transition from being harmless inhabitants or passersby of the intestinal tract to causing clinical disease. This hypothesis may be complemented with the possible existence of strains with differing virulences, with the highly virulent strains triggering diarrheal disease. Differences in virulence between strains have been described for other *Helicobacter* species, such as *Helicobacter hepaticus* (21) and *H. pylori* (7, 12). In fact, 70 to 90% of the population in developing countries carries *H. pylori*, while only 25 to 50% develops gastric disease (7). The actual evolvement into gastric disease depends on bacterial factors, host characteristics, and/or interaction between host and bacterium (12). A well-known phenomenon is that strains possessing the *cagA* gene, a component of the pathogenicity island, are substantially more virulent than strains without *cagA* (12, 14, 15, 17). For *H. pullorum*, very few data are available on the actual virulence markers, despite the increasing number of clinical reports involving this pathogen. The only study dealing with this research area demonstrates the production of the cytolethal distending toxin by *H. pullorum*, which is speculated to play an etiological role in the development of diarrhea (28).

Host factors, such as age, genetic background, and immune response, but also ethnicity and regional factors, might also play a role in the clinical outcome of an *H. pullorum* infection. These all have been discussed for *H. pylori* on numerous occasions (7, 13, 22, 23) and to a lesser extent for *H. hepaticus* (11, 26, 27). A report by Whary et al., who infected different mouse strains with *H. hepaticus*, emphasizes the significance of the host response (27). Various studies on the development of hepatitis, liver cancer, and inflammatory bowel disease in mice infected with *H. hepaticus* demonstrate that a genetic basis for susceptibility to *Helicobacter*-induced disease is of importance. Indeed, differences between mouse strains regarding the de-

velopment of liver disease or inflammatory bowel disease are commonly noticed (11, 24, 25, 26).

In conclusion, this is the first detailed report on the prevalence of *H. pullorum* in both patients with gastrointestinal disease and clinically healthy humans, proving that *H. pullorum* is fairly regularly present in the stools of people belonging to either group. To date, the molecular basis of *H. pullorum* colonization and virulence is poorly understood, and hence, further studies to unlock more of the secrets of the lifestyle of this potential pathogen and its encumbrance for public health are necessary.

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